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10/594,864	11/30/2006	Takashi Shinohara	701067	1275

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EXAMINER
SGAGIAS, MAGDALENE K

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/594,864	SHINOHARA ET AL.	
	Examiner	Art Unit	
	Magdalene K. Sgagias	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 July 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12,15 and 16* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12,15 and 16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *29 September 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's arguments filed 07/29/2009 have been fully considered but they are not persuasive. Claims 1-12, 15-16 are pending and under consideration. Claims 13-14, 17-34 are canceled.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The rejection of claims 1-12, 15-16 under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for producing multipotent germline stem (mGS) cells, which comprises culturing testis cells using medium containing glial cell derived neurotrophic factor (GDNF), wherein the testis cells contain spermatogonial stem cells (SSCs), and wherein the testis cells are derived from a postnatal mouse, and isolating multipotent gremline stem cells expressing SSEA-1, Forsman antigen , β1-integrin, α6-integrin, EpCAM, CD9, EE2 and c-kit markers, does not reasonably provide enablement for producing pluripotent stem cells derived from postnatal testis of all mammalian species including human species, forming the three somatic lineages is maintained for the reasons of record.

Applicants argue the specification describes the problems associated with the production of pluripotent stem cells from adult testis and specific methods to solve the problems. In particular, the specification identifies the problem to be solved in the production of pluripotent stem cells from adult testis cells as low frequencies of stem cells in adult testis. The specification provides specific guidance as to methods of solving the problem including the use

of a younger animal because younger animals have higher frequencies of stem cells (e.g., spermatogonial stem cells) contained in the testis (see, e.g., page 14, line 23, through page 15, line 5, and page 16, line 3-6). Accordingly, one of ordinary skill in the art would understand how to use the inventive methods with a reasonable expectation of success.

These arguments are not persuasive because producing pluripotent stem cells from testis cells derived from a postnatal mammal including younger animals with higher frequencies of stem cells (e.g., spermatogonial stem cells) embrace pluripotent stem cells derived from embryonic stem cells. The postnatal mammal as instantly claimed does not have embryonic stem cells other than spermatogonial stem cells (embryonic germ stem cells) having the same markers as the embryonic stem cells derived fro the ICM. The spermatogonial stem cells acquire markers of the spematogonial lineage while the embryonic stem cells derived from the ICM lack said lineage markers.

Applicants argue post-filing references further evidence the success of the inventive methods described in the specification. In particular, Guan et al., Nature, 440: 1199-1203 (2006), and Guan et al., Nature Protocol, 4:143-154 (2009) (copies of which are submitted herewith), disclose the generation of pluripotent stem cells from normal adult murine testis cells using the inventive methods. Conrad et al., Nature, 456:344-351 (2008), and Kossack et al., Stem Cells, 27:138-149 (2009) (copies of which are submitted herewith), disclose the generation of pluripotent stem cells from normal adult human testis cells using the inventive methods.

These arguments are not persuasive because Guan (2006) teaches the isolation of multipotent adult germline stem cells (maGSCs) which are able to spontaneously differentiate into derivatives of the three embryonic germ layers in vitro and generate teratomas in

immunodeficent mice, wherein those spermatogonial stem cells, express the spermatogonia-specific marker *Stra8* are derived from adult mouse testis (abstract). Those cells taught by Guan are not embryonic stem cells (ESCs) derived from ICM as embraced in the instant invention because unlike the ESC express the spermatogonia-specific marker *Stra8.* Guan teaches the pluripotency of adult SSCs and SSC-derived maGSCs, similar to ESCs, therefore the SSC-derived maGSCs, are not identical to ESCs as embraced in the instant invention. Guan teaches a previous study failed to establish ES-like cells from adult mouse testis referring to the cells of the instant Applicant (emphasis added). Guan teaches the use of a genetic selection to enrich SSCs, which is more efficient than CD9 antibody selection of the Applicant and unlike the Applicant's method fibroblast growth factor (bFGF), epidermal growth factor (EGF) and LIF for the initial culture of testicular cells, growth factors results in the overgrowth of other cells, including fibroblasts, endothelial and Sertoli cells and although GDNF is essential for the self-renewal of SSCs *in vivo* the proliferation of mouse SSCs *in vitro* is not dependent on GDNF but instead on LIF (p 1202, 1st column, last paragraph). Guan teaches SSCs themselves may be multipotent (p 1202, 1st column, last paragraph). Guan suggests when SSCs are expanded in the absence of Sertoli cells, *in vitro* culture and in blastocysts, some SSCs might be released from this inhibition and converted into pluripotent stem cells (p 1202, 1st column, last paragraph). Therefore, Guan does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as instantly claimed.

Regarding Guan (2009) reference like Guan 2006 teaches an a modified method of the isolation of maGSCs and for the same reasons as discussed above does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as instantly claimed.

Regarding Conrad (2009) reference Conrad teaches there are considerable differences between mouse and human ES cells and presumably between adult mouse and human GSCs as well (p 6, 1st column, last paragraph). Conrad teaches the precise molecular and cellular mechanisms that specifically regulate the proliferation and pluripotency of human adult GSCs remain unclear. Therefore, Conrad does not teach the derivation of pluripotent stem cells from testis cells containing ESCs as instantly claimed.

Regarding Kossack (2009) reference Kossack teaches the derivation of human multipotent germline stem cells (hMGSCs) from a testis biopsy (abstract). Kossack suggest the potential to derive pluripotent cells from human testis biopsies but indicate a need for novel strategies to optimize hMGSC culture conditions and reprogramming for the derivation of human multipotent germline stem cells (hMGSCs) from a testis biopsy (abstract). Therefore, Kossack teaches multipotent and not pluripotent cells as instantly claimed.

Applicants argue one of ordinary skill in the art at the time of filing the application would have been able to easily extrapolate the production conditions of human pluripotent stem cells from the production conditions of mouse pluripotent stem cells given the teachings in the prior art, such that no undue experimentation would be required to practice the inventive methods. For example, Hogan (U.S. Patent 5,690,926) demonstrates that pluripotent stem cells can be produced from human primordial germ cells under basically the same conditions as mouse embryonic germ cells (e.g., stem cell factor (SCF), basic fibroblast growth factor (bFGF), and leukemia inhibitory factor (LIF)) (see column 12, lines 14-60). In particular, Hogan describes that the methods of isolation of ES cells from murine embryos were repeated for isolation of ES cells from human embryos (see column 12, lines 17-19).

These arguments are in part convincing for the derivation of pluripotent stem cells from spermatogonial stem cells but not for the derivation of pluripotent stem cells from embryonic stem cells derived from ICM as embraced in the instant invention because the embryonic stem cells lack specific germline markers as the spermatogonial stem cells for example express the spermatogonia-specific marker *Stra8* as discussed above.

Applicants argue Tumpenney et al. (2006) as evidence that human EG cell derivation and culture include the use of additional additives than required for mouse EG cell derivation and culture. Among the additives described in Turnpenney et al. (2006), forskolin was considered to specifically act on human EG cells; however, forskolin also is reported to be effective for mouse EG cells (see Koshimizu et al., Development, 122:1235-1242 (1996); a copy of which is enclosed herewith). While factors (e.g., forskolin) may improve the culture conditions of EG cells of mammals, Applicants note that the culture conditions for human pluripotent stem cells do not differ much from the culture conditions for mouse pluripotent stem cells and that both require the use of GDNF as an essential factor (see, e.g., Hogan, Guan et al. (2006), Guan et al. (2009), Conrad et al., and Kossack et al.).

These arguments are not persuasive because as discussed above for example teaches although GDNF is essential for the self-renewal of SSCs *in vivo* the proliferation of mouse SSCs *in vitro* is not dependent on GDNF but instead on LIF. Guan teaches the use of a genetic selection to enrich SSCs, which is more efficient than CD9 antibody selection of the Applicant and unlike the Applicant's method fibroblast growth factor (bFGF), epidermal growth factor (EGF) and LIF for the initial culture of testicular cells, growth factors results in the overgrowth of other cells, including fibroblasts, endothelial and Sertoli cells. Tumpenney teaches additional issues regarding the culturing of embryonic germ cells from different species as a source of

spermatogonial germ cells as instantly required for the culture of said cells to obtain pluripotent cells. Tumpenney and brings the issue of the differences in pluripotent stem cells between mice and humans, wherein for example, despite activation of the LIFR/gp130-STAT3B pathway, LIF does not maintain self-renewal of hESCs, which require feeder cells or their conditioned media with an extracellular matrix. Therefore, the culture of embryonic germ cells from different species is controversial and unpredictable at best.

Applicants argue Aflatoonian et al. describes the difficulty of maintaining well-defined hEG cell lines through extended passage in culture, even though the initial generation of hEG cells is relatively simple. Applicants note that the pending claims are directed to a method of producing (i.e., generating) pluripotent stem cells from testis cells, and not to a method of maintaining human EG cells in culture over multiple passages. However, even if the maintenance of human EG cells is difficult, Applicants note that human EG cells have been established as described in Turnpenney et al., Stem Cells, 21:598-609 (2003) (a copy of which is submitted herewith).

These arguments are not persuasive because the Turnpenney et al., Stem Cells, 21:598-609 (2003) reference teaches derivation of human embryonic germ cells as an alternative source of pluripotent stem cells from embryos unlike the instantly claimed postnatal derived cells (adult testis cells) (see p 599, under materials and methods).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be

> patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The rejection of claims **1-2, 4-6, 15** under 35 U.S.C. 103 (a) as being unpatentable over **Hogan** (US 5,690,926) in views of **Creemers et al,** (Reproduction, 124: 791-799, 2002) is maintained for the reasons of record.

Applicants argue that Hogan discloses a method of making mammalian pluripotent ES cells by culturing postnatal mammalian testis in a composition comprising bFGF and LIF. The Office also contends that Hogan teaches isolating the ES cells from postnatal mammalian testis. The Office acknowledges that Hogan does not teach the use of GDNF; however, the Office contends that Creemers et al. teaches culturing spermatogonial cells in a medium containing GDNF, LIF, and bFGF. The Office contends that it would have been obvious for one of ordinary skill in the art to add GDNF to the culture system of Hogan because Creemers et al. suggests that optimization of the culture medium could improve viability or proliferation of spermatogonia. Applicants argue the Example of Hogan at column 12; line 61, through column 13, line 13, and this portion of the Example is written in present tense and provides no evidence that the method was actually performed.

In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, Hogan discloses a method of making a mammalian pluripotential embryonic stem cell comprising culturing postnatal mammalian testis in a composition comprising a growth

enhancing amount of basic fibroblast growth factor, leukemia inhibitory factor, membrane associated steel factor, and soluble steel factor, thereby making a pluripotential embryonic stem cell from a germ cell. It would have been prima facie obvious to a person of ordinary skill in the art of obtaining pluripotent stem cells from testis cells to combine the teachings of Creemers by adding GDNF in to the culture medium because Creemers teaches comparison of cultures of adult cells with cultures of prepubertal germ cells, commonly used in studies of spermatogenesis, showed that prepubertal germ cells are twice as viable by adding GDNF to the basic culture media containing bFGF and LIF since Hogan already taught the basic culture media containing bFGF and LIF.

Applicants argue the method described in Hogan is substantially the same as the method of generating EG cells from primordial germ cells disclosed in Matsui et al., Cell, 70(5): 841-847 (1992), which was previously cited by the Office. Brigid L. M. Hogan is listed as the inventor of Hogan and a co-author of Matsui et al. The Office cites to the method for the isolation of embryonic stem cell lines from postnatal mammalian testis set forth in the Example of Hogan at column 12, line 61, through column 13, line 13. Applicants note that this portion of the Example is written in present tense and provides no evidence that the method was actually performed.

These arguments are not persuasive because the obviousness is based on the teachings, suggestion and motivation of the combined references as discussed above in the case of the combined references of Hogan taken with Creemers and not on evidence that the method was actually performed.

Applicants argue Labosky et al. (Development, 12:3197-3204 (1994)), which is a later-published reference from the same laboratory as the inventor of Hogan, reports that while pluripotent cell lines can be established from primordial germ cells of 8 days post coitum (p.c.)

embryos and 12.5 days p.c. genital ridges, germ cells from the gonads of 15.5 days p.c. embryos and newborn mice (i.e., postnatal germ cells) did not give rise to embryonic germ cell lines under the conditions disclosed in the prior art (see, e.g., page 3199, paragraph bridging columns 1 and 2). Applicants note that Labosky et al. references experiments described in Matsui et al. which are the substantially the same as those described in Hogan. Therefore, one of ordinary skill in the art would have concluded from a consideration of the prior art disclosures (including Labosky et al.) that Hogan does not teach that pluripotent stem cells can be established from postnatal cells, as required by the pending claims.

These arguments are not persuasive because obviousness is based on the teachings, suggestion and motivation of the combined references. In the instant case, even though Labosky teaches 15.5 days p.c. embryos and newborn mice (i.e., postnatal germ cells) did not give rise to embryonic germ cell lines under the conditions disclosed in the prior art, however, Hogan discloses a method of making a mammalian pluripotent embryonic stem cell comprising culturing postnatal mammalian testis in a composition comprising a growth enhancing amount of basic fibroblast growth factor, leukemia inhibitory factor, membrane associated steel factor, and soluble steel factor, thereby making a pluripotent embryonic stem cell from a germ cell. Thus, it would have been prima facie obvious to an ordinary skilled artisan to combine the teachings of Creemers for the same reasons as discussed above.

Applicants argue the inventors recognized that pluripotent stem cells could be established from postnatal mammals without destruction of embryos or genetic modification using the inventive method. The inventive method circumvents the ethical problem of destroying embryos in the production of pluripotent stem cells, which is surprising and unexpected in view of the methods disclosed in the prior art (see, e.g., Labosky et al.).

These arguments are not persuasive because the ethical issues are irrelevant to the obviousness type of rejections for the same reasons as discussed above.

The rejection of claim **3** under 35 U.S.C. 103(a) as being unpatentable over **Hogan** (US 5,690,926) in view of **Creemers et al,** (Reproduction, 124: 791-799, 2002) as applied to claims **1-2, 4-6, 15** above, and further in view of **Haneji et al** (J Endorinology, 128(3): 383-8, 1991 taken with **Wahab-Wahlgren** (Mol and Cell Endocrin, 201: 39-46, 2003) is maintained for the reasons of record.

Applicants have failed to respond to the above rejection.

The rejection of claim **7** under 35 U.S.C. 103(a) as being unpatentable over **Hogan** (US 5,690,926) in view of **Creemers et al,** (Reproduction, 124: 791-799, 2002) as applied to claims **1-2, 4-6, 15** above, and further in view **Beumer et al** (Cell Death and Differentiation , 5: 669-677, 1998 (IDS)) is maintained for the reasons of record.

Applicants have failed to respond to the above rejection.

The rejection of claim **16** under 35 U.S.C. 103(a) as being unpatentable over **Hogan** (US 5,690,926) taken with **Creemers et al,** (Reproduction, 124: 791-799, 2002) and further in view of **Kanatsu-Shinohara et al, [**Biology of Reproduction 70, 70–75 ,2004, (IDS)]; **Shinohara et al,** [PNAS, 96: 5504-5509, 1999, (IDS)]; **Van Der Wee et al** (Journal of Andrology, 22(4): 696-704, 2001) is maintained for the reasons of record.

Applicants have failed to respond to the above rejection.

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

Magdalene K. Sgagias
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/Anne-Marie Falk, Ph.D./
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